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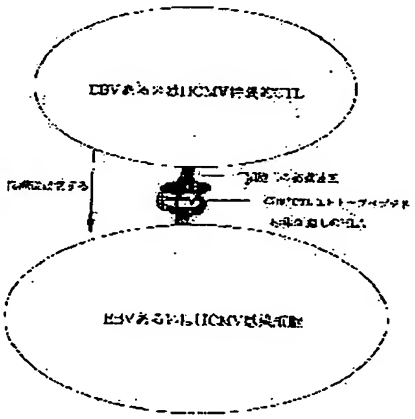
(54) CD8+ CYTOTOXIC T LYMPHOCYTE EPITOPE PEPTIDE AND ITS USE

(57)Abstract:
PROBLEM TO BE SOLVED: To obtain a CD8+ cytotoxic T lymphocyte epitope peptide specific to an Epstein-Barr virus or human cytomegalovirus.
SOLUTION: This CD8+ cytotoxic T lymphocyte epitope peptide is specific to an Epstein-Barr virus or human cytomegalovirus.



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CLAIMS DETAILED DESCRIPTION TECHNICAL FIELD PRIOR ART EFFECT OF THE INVENTION TECHNICAL PROBLEM MEANS EXAMPLE DESCRIPTION OF DRAWINGS DRAWINGS

[Translation done.]

CLAIMS

[Claim(s)]

[Claim 1] A CD8+ cell trauma nature T lymphocyte epitope peptide specific to an Epatein-Barr virus or a human cytomegalovirus.

[Claim 2] A peptide according to claim 1 which is a thing including at least one amino acid sequence chosen from a group to which a specific CD8+ cell trauma nature T lymphocyte epitope peptide becomes an Epatein-Barr virus from an array number 1 - an array number 22.

[Claim 3] A peptide according to claim 1 which is a thing including at least one amino acid sequence chosen from a group to which a specific CD8+ cell trauma nature T lymphocyte epitope peptide becomes a human cytomegalovirus from an array number 23 - an array number 32.

[Claim 4] A vaccine for treating or preventing infection of an Epatein-Barr virus which contains a peptide according to claim 2 as an active principle.

[Claim 5] A vaccine for treating or preventing infection of a human cytomegalovirus which contains a peptide according to claim 3 as an active principle.

[Claim 6] A vaccine for treating or preventing infection of an Epatein-Barr virus which contains an antigen presenting cell which carried out the pulse of the peptide according to claim 2 as an active principle.

[Claim 7] A vaccine for treating or preventing infection of a human cytomegalovirus which contains an antigen presenting cell which carried out the pulse of the peptide according to claim 3 as an active principle.

[Claim 8] A passive immunity therapy agent to an Epatein-Barr virus or a human cytomegalovirus containing a CD8+ cell trauma nature T lymphocyte which stimulates a peripheral blood lymphocyte by antigen presenting cell which carried out the pulse of a peptide according to claim 1 to 3 or this peptide, and is obtained.

[Claim 9] A passive immunity therapy agent to an Epatein-Barr virus or a human cytomegalovirus which major histocompatibility antigen complex prepared from a peptide according to claim 1 to 3 and/or a major histocompatibility antigen complex-tetramer, and a peripheral blood lymphocyte are made to react, is made to form combination which a CD8+ cell trauma nature T lymphocyte combined with this major histocompatibility antigen complex and/or a major histocompatibility antigen complex-tetramer, and contains a CD8+ cell trauma nature T lymphocyte which isolates and is obtained from this combination.

[Claim 10] A passive immunity therapy agent to an Epatein-Barr virus or a human cytomegalovirus which a major histocompatibility antigen complex-indicator MAG bead and a peripheral blood lymphocyte which were prepared from a peptide according to claim 1 to 3 are made to react, is made to form combination which a CD8+ cell trauma nature T lymphocyte combined with a major histocompatibility antigen complex-indicator MAG bead, and contains a CD8+ cell trauma nature T lymphocyte which isolates and is obtained from this combination.

[Claim 11] A quantum method of a CD8+ cell trauma nature T lymphocyte specific to an Epatein-Barr virus or a human cytomegalovirus characterized by measuring cytokine and/or a chemokine which stimulate peripheral blood with a peptide according to claim 1 to 3, and obtain a specific CD8+ cell trauma nature T lymphocyte with this virus, and this CD8+ cell trauma nature T lymphocyte produces.

[Claim 12] A quantum method of a CD8+ cell trauma nature T lymphocyte specific to an Epatein-Barr virus or a human cytomegalovirus in this peripheral blood to which prepare a major histocompatibility complex and/or major histocompatibility complex-tetramer from a peptide according to claim 1 to 3, and a this major histocompatibility complex and/or major histocompatibility complex-tetramer and peripheral blood are made to react.

[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[The technical field to which invention belongs] This invention is an Epatein-Barr virus (Epstein-Barr virus). Hereafter, or it calls EBV, it is a human cytomegalovirus (human cytomegalovirus). A CD8+ cell trauma nature T lymphocyte specific for calling HCMV hereafter (cytotoxic T lymphocyte) It is related with the quantum method of CD8+CTL specific to the passive immunity therapy agent to the vaccine, EBV, or HCMV which treats or prevents infection of EBV using the epitope peptide and this peptide which are called CTL, or HCMV hereafter and EBV, or HCMV.
[0002]
[Description of the Prior Art] EBV has relation deeply with the onset of a Burkitt lymphoma and a nasopharyngeal carcinoma, and relation with a Hodgkin disease, gastric cancer, etc. attracts attention. Moreover, it is important as main cause viruses of the opportunism lymphoma which has been a problem after a congenital immune deficiency, the acquired immunode-ficiency syndrome patient, or the organ transplantation etc., and the oppression influences a patient's prognosis greatly.
[0003] On the other hand, HCMV is the most important pathogen as a cause virus of the opportunistic infection which has been a problem after the acquired immunode-ficiency syndrome patient or the organ transplantation etc. Moreover, the hematopoietic stem cell transplantation between non-blood relationship persons and using cord blood is increasing remarkably by expansion of a bone marrow bank and a cord blood bank in recent years. Since immunosuppression more powerful than usual is carried out in such transplantation for refusal prevention, the opportunistic infection which makes HCMV representation occurs in high frequency, and not only serious-illness-izing but the example of death does not have it. [rare]
[0004] In such a medical situation and the social background, the new method of controlling EBV and HCMV safely and effectively is desired strongly. The main immunocompetent cells which are controlling the activity of EBV and a HCMV infected cell are CD8+CTL(s). Since CD8+CTL has the capacity which destroys it if EBV and a HCMV infected cell are discovered, if the function is activated effectively, its a possibility of leading to development of a new diagnosis of EBV and CMV associated diseases and a cure is high.
[0005] In case CTL recognizes a virus infection cell, there are the following features. (1) CTL cannot recognize the virion itself. (2) CTL recognizes the peptide (an epitope peptide is called hereafter) which consists of 9-10 amino acid in the specific part in virus protein, and destroys an infected cell. (3) It combines with the human leukocyte antigen (HLA is called human leucocyte antigen and the following) in virus infection cell surface, and CTL is shown this 9-10 specific amino acid. (4) When HLA changes with individual human beings between races and HLAs differ, also in the same virus, epitope peptides differ.
[0006] It can become an indispensable matter at a diagnosis of whether there is any effective immunity to these viral infectious diseases, and the time of giving an immunity cure further to determine the amino acid which can serve as a specific epitope peptide as EBV and HCMV the neither more nor less so that clearly from having stated by (1) to (4) of a more than. However, HLA which Japanese [many of] holds About a mold, there are very few reports of a CD8+CTL epitope peptide specific to these EBV or HCMV(s), and treating or preventing infection of EBV or HCMV is asked for development of a useful epitope peptide.
[0007]
[Problem(s) to be Solved by the Invention] This invention aims at providing with the specific quantum method of CD8+CTL the passive immunity therapy agent to the vaccine, EBV, or HCMV which treats or prevents infection of a specific CD8+CTL epitope peptide, EBV, or HCMV to EBV and HCMV and EBV, or HCMV.
[0008]
[Means for Solving the Problem] In order to solve the above-mentioned technical problem, as a result of inquiring wholeheartedly, this invention persons find out two or more epitope peptides which CD8+CTL which controls EBV and HCMV can recognize, and came to complete this invention. That is, this invention is a CD8+CTL epitope peptide specific to EBV and HCMV.
[0009] Moreover, this invention is a vaccine for treating or preventing infection of EBV, EBV using a CD8+CTL epitope peptide specific to HCMV, or HCMV. Furthermore, this invention is a passive immunity therapy agent to EBV or HCMV prepared using this peptide. Moreover, this invention relates to a quantum method of CD8+CTL specific to EBV or HCMV.
[0010]
[Embodiment of the Invention] This invention is further explained to details.
1. The peptide as used in the field of epitope peptide this invention means the chain of the linear amino acid which has

bioactive and combined it mutually by the peptide linkage between alpha-amino group of the adjoining amino acid residue, and a carboxyl group. A peptide does not mean specific merit's thing and may be various length. Moreover, you may be the gestalt of a non-charge or a salt, and it may be embellished by glycosylation, amidation, phosphoryl-izing, carboxylation, phosphorylation, etc. depending on the case. Furthermore, when the bioactive of the epitope of this invention and immunity activity are not changed substantially but are prescribed for the patient, unless it has harmful activity, the peptide which one piece or insertion of amino acid [some (for example, 1-10 pieces)], addition, substitute, etc. produced is also contained in this invention. For example, that to which it is placed between the amino terminals or C terminals of a peptide by the additional amino acid sequence is also contained. Moreover, the peptide of this invention can be used as a gestalt of the derivative by complex, radioisotope, etc. with which the saccharide, the polyethylene glycol, the lipid, etc. were added, or a polymer.

[0011] In CD8+CTL of this invention, the CD8+ cell trauma nature T lymphocyte which has discovered CD8 which is one of the surface antigen molecules which exist on a Homo sapiens lymphocyte is meant. With moreover, a specific CD8+CTL epitope peptide, to EBV or HCMV It is the peptide which consists of 9-10 amino acid sequences in the specific part in EBV which may be recognized by CD8+CTL, or HCMV protein. It is a part for the structured division of EBV combined with the antigen acceptor of CD8+CTL in immunity, or HCMV, and they are EBV or HCMV. The trauma of the infected cell is carried out directly, and the antigen radical which activates the cellular immunity device of CD8+CTL which can eliminate a virus is meant. The device is shown in drawing 1 .

[0012] A CD8+CTL epitope peptide specific to EBV or HCMV of this invention Collating data medium with which the epitope peptide which consists of 9-10 amino acid which has the joint motif of HLA-A24 and A26 grade about the amino acid sequence of EBV or HCMV protein can be searched, For example BioInformatics & Molecular Analysis Section currently exhibited on the Internet (BIMAS) HLA Peptide Binding Predictions It can collate by (http://bimas.dcrt.nih.gov/molbio/hla_bind/index.html.) and an epitope peptide and the peptide (an epitope candidate peptide is called hereafter) which can become can be screened.

[0013] The peptide which has the amino acid sequence of the array numbers 1-32 of this invention is checked as a CD8+CTL epitope peptide as a result of the above screening. That is, the epitope peptide shown in the array numbers 1-32 of this invention may be prepared by various kinds of conventional peptide synthesis methods. For example, it is also possible to prepare organic chemistry-synthesis methods, such as a solid-phase-peptide-synthesis method, or DNA which carries out the code of the peptide, and to prepare using recombinant DNA technology. Moreover, composition by commercial chemosynthesis equipment (for example, peptide synthesizer unit of an applied biotechnology systems company) is also possible.

[0014] An epitope candidate peptide can be obtained by the usual chemosynthesis. About the obtained epitope candidate peptide, it can determine whether it is a specific CD8+CTL epitope peptide as EBV or HCMV with either of the methods as shown below.

[0015] (1) It is 2x106-/ml to 110% fetal-calf-serum content RPMI1640 of the epitope peptide decision methods culture medium. The lymphocyte separated from the adult infected with EBV or HCMV by cell concentration is made to float, and it mixes with EBV or 1x105/ml of HCMV infected cells which carried out isolation culture of this lymphocyte beforehand from the same people, and cultivates for ten days at 37 degrees C with a carbon-dioxide-gas thermostat. Specific CD8+CTL is guided to EBV or HCMV by adding interleukin 2 (IL-2 being called hereafter) on the 10th day of culture, and repeating EBV or a HCMV infected cell, and the stimulus by IL-2 once per week henceforth. Thus, it judges whether it has the capacity which an epitope candidate peptide stimulates to guided CD8+CTL by ERIS pot assay etc. ERIS pot assay is reported in work besides Kuzushima K, The Journal of Clinical Investigation, 104 : 163 - 171 pages, 1999, etc.

[0016] (2) Float the lymphocyte separated into 210% fetal-calf-serum content RPMI1640 of the epitope peptide decision methods culture medium from the adult infected with EBV or HCMV by 2x106-/ml cell concentration, and add one sort of the arbitration in an epitope candidate peptide to this by 1microg [/ml] concentration. It cultivates for ten days at 37 degrees C with a carbon-dioxide-gas thermostat. Specific CD8+CTL is guided by adding IL-2 on the 10th and repeating said peptide and stimulus by IL-2 once per week henceforth. Thus, it judges whether an epitope candidate peptide stimulates to specific CD8+CTL to guided EBV or HCMV by ERIS pot assay etc.

[0017] (3) Compound the peptide library which consists of amino acid around 20 pieces which cover epitope peptide decision method 3EBV or the whole HCMV protein. To the compound peptide, it is made short one by one about the peptide to which CD8+CTL reacted, and the epitope peptide which finally consists of 9-10 amino acid is obtained, and it considers as the epitope peptide of this invention.

[0018] 2. The CD8+CTL epitope peptide of vaccine this invention can be used as a vaccine in an active immunity peptide vaccine therapy. That is, a patient can be medicated with the vaccine which comes to contain the CD8+CTL epitope peptide of this invention, EBV or HCMV can be made to be able to increase specific CD8+CTL in a body, and it can use for the prevention and the therapy to a disease. Even if the epitope peptides to be used are one sort of use, they can also be used according to the purpose of using a vaccine, being able to combine two or more sorts of peptides, and mixing.

[0019] Moreover, the vaccine containing what carried out the pulse of the CD8+CTL epitope peptide of this invention to antigen presenting cells (for example, a dendrite cell, a B cell, a macrophage, etc.) (a CD8+CTL epitope peptide pulse cell is called hereafter) can also be used. Here, an antigen presenting cell means mixing this peptide with an antigen presenting cell from 30 minutes in suitable culture medium for 1 hour as meaning and carrying out the pulse of what has CD8+CTL stimulus ability in the cell which discovers HLA which this peptide can combine on the surface.

[0020] The CD8+CTL epitope peptide of this invention or the vaccine which comes to contain a CD8+CTL epitope peptide pulse cell can be prepared using a well-known method in a field for the time being. For example, as this

vaccine, there is injections or a solid preparation etc. which contains a CD8+CTL epitope peptide [of this invention] or CD8+CTL epitope peptide pulse cell as an active principle. As a salt which can prescribe an epitope peptide with the gestalt of neutrality or a salt, for example, may be permitted on pharmaceutical sciences, organic acids, such as mineral salt of a hydrochloric acid, a phosphoric acid, etc. or an acetic acid, and a tartaric acid, are mentioned. Moreover, the epitope peptide of this invention or an epitope peptide pulse cell is permitted on medicine manufacture, can mix with such combination the excipient which has the activity and compatibility of this peptide or this cell, for example, water, brine, glucose, ethanol, glycerol, DMSO, other adjuvants, etc., and can be used. Furthermore, adjuvants, such as albumin, a wetting agent, and an emulsifier, may be added if needed.

[0021] Although the vaccine of this invention can be prescribed for the patient by parenteral administration and internal use, generally its parenteral administration is desirable. As parenteral administration, there are injections, such as subcutaneous injection, an intramuscular injection, and an intravenous injection, a suppository, etc. Moreover, as internal use, it can prepare as mixture with excipients, such as starch, a mannitol, a lactose, magnesium stearate, and a cellulose.

[0022] The vaccine of this invention is prescribed for the patient in an effective amount on a therapy. The dose which needs the amount prescribed for the patient depending on the immune system for a therapy is determined by decision of a clinician. Usually, let a suitable dose be the content of 106-109 pieces in 1-100mg and an epitope peptide pulse cell per patient and with an epitope peptide. Moreover, an administration gap can be set up for an object and the purpose.

[0023] 3. The CD8+CTL epitope peptide of passive immunity therapy agent this invention can be used for preparation of the passive immunity immunity therapy agent to EBV or HCMV. Namely, CD8+CTL which stimulates 1 peripheral blood lymphocyte with this peptide, and is obtained, 2) Major histocompatibility antigen complex which prepared the peripheral blood lymphocyte from this peptide (major histocompatibility complex) Or it calls MHC, make it react with an MHC-tetramer and the combination which CD8+CTL combined with the MHC or MHC-tetramer is made to form hereafter. CD8+CTL which isolates and is obtained from this combination, Or it can be made to be able to react with the MHC-indicator MAG bead which prepared 3 peripheral blood lymphocytes from this peptide, the combination which CD8+CTL combined with the MHC-indicator MAG bead can be made to be able to form, and the passive immunity immunity therapy agent containing CD8+CTL which isolates and is obtained from this combination can be obtained.

[0024] The MHC and MHC-tetramer which used the CD8+CTL epitope peptide can be prepared as follows, for example. MHC which is the complex of the HLA heavy chain refined from the Escherichia coli for a protein manifestation, beta 2-microglobulin, and the CD8+CTL epitope peptide of this invention is made to form within a buffer. The biotin bonding site is beforehand added to the C terminal of recombination HLA heavy chain protein, and a biotin is added to this part after MHC formation. An MHC-tetramer is produced by mixing commercial indicator coloring matter streptoavidin and commercial biotin-ized MHC by the mole ratio 1:4. The MHC-tetramer obtained by drawing 2 and drawing 3 and its reaction mechanism are shown, respectively. In addition, in each step, it is desirable to perform protein purification by gel filtration. CD8+CTL specific to EBV or HCMV contained in a passive immunity therapy agent can be obtained by the following preparation methods.

[0025] (1) Separate CD8+CTL preparation method 1 lymphocyte from peripheral blood etc., and make this react for 15 minutes 37 degrees C with MHC of suitable concentration, or an MHC-tetramer. Since specific CD8+CTL is dyed EBV or HCMV combined with the MHC or MHC-tetramer by indicator coloring matter, only CD8+CTL dyed using flow cytometer, a microscope, etc. is isolated. Or the solid-phase-ized MHC and/or MHC-tetramer can also be made to react to a sterile plate etc. beforehand. EBV combined with the MHC and/or MHC-tetramer solid-phase-ized by the plate, or HCMV -- specific -- the antigen which remained on the plate after flushing other cells which are floating without joining together, in order to isolate CD8+CTL -- specific -- only CD8+CTL is suspended in new culture medium. Thus, CD8+CTL specific to EBV or HCMV by which isolation was carried out carries out stimulus growth by the T cell stimulant agent of anti-CD3 antibody, PHA, and IL-2 grade, and secures the number of cells required for a passive immunity therapy.

[0026] (2) the CD8+CTL preparation method 2 -- combine with a streptoavidin indicator MAG bead biotin-ized MHC prepared from the peptide as mentioned above, and produce combination (an MHC-MAG bead is called hereafter). An MHC-MAG bead is shown in drawing 4 . A lymphocyte is separated from peripheral blood etc. and said MHC-MAG bead of suitable concentration is made to react by the lymphocyte:bead ratio and 1:10. CD8+CTL which combined with the bead the test tube with which specific CD8+CTL went into EBV or HCMV combined with the MHC-MAG bead also in the magnetic field is brought near by the test tube wall of a side with a magnet. The device is shown in drawing 5 . Then, after flushing the other cell, a test tube is removed from a magnetic field and only antigen-specific CD8+CTL which remained in the test tube wall is suspended in new culture medium. Thus, CD8+CTL specific to EBV or HCMV by which isolation was carried out carries out stimulus growth by the T cell stimulant agent of anti-CD3 antibody, PHA, and IL-2 grade, and secures the number of cells required for a passive immunity therapy.

[0027] (3) Stimulate by the antigen presenting cells (for example, a dendrite cell, a B cell, a macrophage, etc.) which carried out the direct stimulus of the lymphocyte separated from CD8+CTL preparation method 3 peripheral blood with the CD8+CTL epitope peptide of this invention, or carried out the pulse of this peptide. CD8+CTL guided by the stimulus will be cultivated at 37 degrees C with a carbon-dioxide-gas thermostat for seven to ten days. After that IL-2 are added and CD8+CTL of the number of cells required for a passive immunity therapy is secured by repeating the stimulus by the CD8+CTL epitope peptide, IL-2, or this antigen presenting cell and IL-2 once per week. Suspension of the CD8+CTL specific to EBV or HCMV obtained as mentioned above can be carried out to the Homo sapiens albumin content PBS etc., and it can be made into the passive immunity therapy agent to EBV or HCMV.

[0028] 4. Specific CD8+CTL in Quanta EBV and HCMV of CD8+CTL a high-risk patient (the man and innate immunity insufficiency to which immunological competence fell according to a certain cause --) The patient who has received administration of an immunosuppresant in response to hematopoietic stem cell transplantation or a solid organ transplantation for refusal prevention, It is important information including proper use of an antivirotic or an immunosuppresant to get to know whether it exists in peripheral blood, such as a chronic viral infectious disease patient, an acquired immunode-ficiency syndrome patient, elderly people, ******, and a gravida, on these infectious disease managements. The specific quantum of CD8+CTL can be performed to EBV or HCMV by the two methods of the following which used the CD8+CTL epitope peptide of this invention.
[0029] The 1st quantum method is the method of carrying out the quantum of the cytokine and/or the chemokines which CD8+CTL guided by stimulating the lymphocyte separated from peripheral blood with the CD8+CTL epitope peptide of this invention produces, such as interferon gamma (IFNgamma) and interleukin. Taking the case of IFNgamma, a method is shown concretely below..
[0030] (1) The method 1 (intracellular IFNgamma production cell quantum) by the cytokine quantum; make the lymphocyte separated from peripheral blood float by 2x106-/ml cell concentration to fetal-calf-serum content RPMI1640 culture medium 10%, and add the CD8+CTL epitope peptide of this invention by 1microg [/ml] concentration. Brefeldin A which is furthermore an intracellular protein transportation inhibition agent is added, and it cultivates at 37 degrees C with a carbon-dioxide-gas thermostat for 5 to 6 hours. Immobilization and film transparency processing are performed and a cell is made to react with a coloring matter indicator anti-IFNgamma antibody and anti-CD8 antibody after culture. The quantum of the IFNgamma positivity cell % in a CD8+ lymphocyte is carried out using flow cytometer etc.
[0031] (2) Wind around each hole the lymphocyte separated from peripheral blood after coating the method 2(ERIS pot assay);96 hole MultiScreen-HA plate (Mi1lipore) by the cytokine quantum with 4 degrees C by the anti-IFNgamma monoclonal antibody overnight and washing each hole by PBS. An epitope peptide is put into each hole and it cultivates in 37-degree C 5% CO2 incubator for 20 hours. after washing a plate by the 0.05%Tween-20 addition PBS on the next day, it is made to each every 90 react at a room temperature in order of an anti-IFNgamma rabbit blood serum and a peroxidase-labeling anti-rabbit IgG goat blood serum 3-amino-9-ethylcarbasole (Sigma) and the 0.1M sodium acetate buffer (pH 5.0) containing 0.015% of H2O2 are put into each hole, and are made to react to a pan at a room temperature for 40 minutes. An IFNgamma spot is visualized and it counts with a stereoscopic microscope.
[0032] (3) The method 3 (how to carry out the quantum of IFNgamma secreted in the culture supernatant) by the cytokine quantum; make the lymphocyte separated from peripheral blood float by 2x106-/ml cell concentration to fetal-calf-serum content RPMI1640 culture medium 10%, and add the CD8+CTL epitope peptide of this invention by 1microg [/ml] concentration. It cultivates at 37 degrees C with a carbon-dioxide-gas thermostat for 24 to 48 hours. ELISA of marketing of the IFNgamma concentration which collects supernatant liquid after culture and is contained in it A quantum is carried out using a kit (for example, HUMAN IFN gamma ELISA of ENDOGEN).
[0033] The quantum of the specific CD8+CTL can be carried out to EBV and HCMV in peripheral blood using the MHC-tetramer produced as the 2nd quantum method using the CD8+CTL epitope peptide of this invention. Preparation of an MHC-tetramer is as above-mentioned. A quantum is the following, and can be made and carried out. A lymphocyte is separated from peripheral blood etc. and 37 degrees C is made to react with the MHC-tetramer of suitable concentration for 15 minutes. Since CD8+CTL combined with this tetramer is dyed by indicator coloring matter, it counts using flow cytometer, a microscope, etc.
[0034]
[Example] Although an example is shown below and this invention is concretely explained to it, this invention is not limited to these.
[Example 1] A CD8+CTL epitope peptide specific to EBV of screening this invention of a CD8+CTL epitope candidate peptide specific to EBV The epitope peptide which consists of 9-10 amino acid which has HLA-A24 and each joint motif of A26 and B61 about the amino acid sequence of EBV protein can be searched. HLA Peptide Binding Predictions of BioInformatics & Molecular Analysis Section (BIMAS) currently exhibited on the Internet () [http:] It collates by //bimas.dcrt.nih.gov/molbio/hla_bind/index.html. Many antigen epitope candidate peptides which consist of 9-10 amino acid which has HLA-A24 and each joint motif of A26 and B61 were screened, and about 100 sorts of epitope candidate peptides were compounded.
[0035] [example 2] **** of the preparation (1) blood of the identification 1. various materials of a CD8+CTL epitope peptide specific to EBV -- the lymphocyte was separated from the peripheral blood of the health adult who has already received infection of EBV. The HLA mold was measured using the serum test.
[0036] (2) The supernatant liquid (a raw EBV virus is included) of B95-8 cell which is an EBV production cell strain was infected with the EBV infection B cell stock peripheral blood lymphocyte, and the EBV infection B cell stock (the EBV infection LCL is called Lymphoblastoid cell line and the following) was established.
[0037] (3) HLA-A24, A26, and B61 gene were respectively introduced into 174CEM.T2 (T2 cell is called hereafter) as a cell for the cell peptide presentation for peptide presentation, and the cell (T2-A24, T2-A26, and T2-B61 cell are called respectively hereafter) which presents HLA-A24, A26, and B61 each joint peptide was obtained. each T -- 2-A24, T2-A26, and T2-B61 cell wer cultivated using Iscove's modified Dulbecco's medium (GIBCO) which added fetal calf serum, L-glutamine, penicillin, streptomycin, and GENECHISHIN 10%.
[0038] 2. The mixed culture of the peripheral blood lymphocyte acquired by specific CD8+CTL aforementioned 1. (1) was carried out to the CD8+ establishment [culture] (1) EBV of CTL specific to EBV within the flask with said LCL of 1. (2) beforehand established from the same people. In this case, it was made not to increase by irradiating the

radiation of the amount of cells lethal at LCL. The antigen epitope which becomes some HLA-A24 which has the feeder of blood in the man's LCL cell surface to HLA-A24, A26, or B61 in a positive case, A26, or B61 molecules from 9-10 amino acid of the EBV protein origin was shown, and CD8+CTL reacted to it started growth. In order to help growth of CD8+CTL, IL-2 were added to culture medium.
(2) From the specific CD8+CTL stock, the CD8+CTL clone was established at EBV in which the specific CD8+CTL clone carried out establishment establishment using limiting dilution to EBV.
[0039] 3. The ERIS pot assay 96 hole MultiScreen-HA plate (Millipore) was coated with 4 degrees C by the anti-IFNgamma monoclonal antibody (R&D Systems, Minneapolis, MN) overnight. After washing each hole by PBS, each cell of T2-A24, and A26 and B61 was wound around each hole. After putting each epitope candidate peptide compounded in the example 1 into each hole and putting at a room temperature for 30 minutes, specific CD8+CTL was added to a suitable number of EBV, and it cultivated in the 37-degree C 5% CO2 incubator for 20 hours. CD8+CTL reacted to an epitope candidate peptide secreted IFNgamma to this incubation period. after washing a plate by the 0.05%Tween-20 addition PBS on the next day, it was made to each every 90 react at a room temperature in order of an anti-IFNgamma rabbit blood serum (Genzyme, Cambridge, MA) and a peroxidase-labeling anti-rabbit IgG goat blood serum Put 3-amino-9-ethylcarbasole (Sigma) and 0.1 M sodium acetate buffer (pH 5.0) containing 0.015% of H2O2 into each hole, it was made to react to a pan at a room temperature for 40 minutes, and the IFNgamma spot was visualized. The spot was counted with the stereoscopic microscope.
[0040] In order to check that the process of the CD8+CTL epitope peptide is carried out to endogenous, the vaccinia virus which carries out the code of each protein of EBV was infected with HLA-A24, A26, and the skin fibroblast of B61 positivity, and ERIS pot assay which made this the antigen presenting cell was performed similarly. Here, the virus protein newly made from infection intracellular as the process is carried out to endogenous is cut by the antigen peptide of right length according to the antigen-processing device which a cell originally has, forms an HLA heavy chain, beta 2-microglobulin, and complex, and means the thing in the condition of being shown to infection cell surface. According to the above-mentioned method, the CD8+CTL epitope peptide of the following array numbers 1-22 with which specific CD8+CTL and its CD8+CTL clone reacted to EBV was obtained.
[0041]
Arg Tyr Ser Ile Phe Phe Asp Tyr Met (array number 1)
Leu Tyr Ala Leu Ala Leu Leu Leu Leu (array number 2)
Ala Tyr Arg Arg Arg Trp Arg Arg Leu (array number 3)
Arg Tyr Cys Cys Tyr Tyr Cys Leu Thr Leu (array number 4)
Thr Tyr Pro Val Leu Glu Glu Met Phe (array number 5)
Ser Tyr Lys Thr Leu Arg Glu Phe Phe (array number 6)
Asp Tyr Asn Phe Val Lys Gln Leu Phe (array number 7)
Thr Tyr Thr Ser Gly Glu Ala Cys Leu (array number 8)
Cys Tyr Glu Asn Asp Asn Pro Gly Leu (array number 9)
Thr Tyr Trp Gln Leu Asn Gln Asn Leu (array number 10)
Ala Tyr Ala Glu Ala Thr Ser Ser Leu (array number 11)
Asp Tyr Met Ala Ile His Arg Ser Leu (array number 12)
Phe Tyr Arg Ser Leu Leu Thr Ile Leu (array number 13)
His Tyr Gln Thr Leu Cys Thr Asn Phe (array number 14)
Phe Tyr Met Thr His Gly Leu Gly Thr Leu (array number 15)
Gly Glu Thr Ser Gly Ile Arg Arg Ala (array number 16)
Asp Leu Ser Tyr Ile Lys Ser Phe Val (array number 17)
Asp Tyr Ser Gln Gly Ala Phe Thr Pro Leu (array number 18)
Leu Tyr Leu Gln Gln Asn Trp Trp Thr Leu (array number 19)
Ile Tyr Val Leu Val Met Leu Val Leu (array number 20)
Ile Tyr Val Leu Val Met Leu Val Leu Leu (array number 21)
Ser Tyr Ala Ala Ala Gln Arg Lys Leu (array number 22)
[0042] [Example 3] A CD8+CTL epitope peptide specific to HCMV of screening this invention of a CD8+CTL epitope candidate peptide specific to HCMV The epitope peptide which consists of 9-10 amino acid which has HLA-A24 and each joint motif of A26 and B61 about the amino acid sequence of HCMV protein can be searched. HLA PeptideBinding Predictions of BioInformatics & Molecular Analysis Section (BIMAS) currently exhibited on the Internet () [http:] It collates by //bimas.dcrt.nih.gov/molbio/hla_bind/index.html. Many antigen epitope candidate peptides which consist of 9-10 amino acid which has HLA-A24 and each joint motif of A26 and B61 were screened, and about 100 sorts of epitope candidate peptides were compounded.
[0043] [example 4] **** of the preparation (1) blood of the identification 1. various materials of a CD8+CTL epitope peptide specific to HCMV, and skin fibroblast -- the lymphocyte was separated from the peripheral blood of the health adult who has already received infection of HCMV. The HLA mold was measured using the serum test. About some men, in order to obtain fibroblast, the biopsy of the skin was performed. That is, after cutting a skin biopsy organization finely with scissors, culture establishment of the skin fibroblast was carried out using 10% fetal calf serum, L-glutamine, BENISHIRIN, and Dulbecco's modified Eagle medium (GIBCO, Grand Island.NY) that added streptomycin.
(2) CMV AD169 strainCMV AD169 strain came to hand from American Type Culture Collection (ATCCVR-538).
[0044] (3) HLA-A24, A26, and B61 gene were respectively introduced into 174CEM.T2 (T2 cell is called hereafter) as a cell for the cell peptide presentation for peptide presentation, and the cell (T2-A24, T2-A26, and T2-B61 cell are

called respectively hereafter) which presents HLA-A24, A26, and B61 each joint peptide was obtained. each T -- 2-A24, T2-A26, and T2-B61 cell were cultivated using Iscove's modified Dulbecco's medium (GIBCO) which added fetal calf serum, L-glutamine, penicillin, streptomycin, and GENECHISHIN 10%.

[0045] 2. Mixed culture was carried out to what infected HCMV with HCMV at the skin fibrocyte of said 1. (1) which established beforehand the peripheral blood lymphocyte acquired by specific CD8+CTL aforementioned 1. (1) to the specific CD8+ establishment [culture] (1) HCMV of CTL from the same people within the flask. The antigen epitope which becomes some HLA-A24 which have the feeder of blood in the man's skin fibrocyte surface to HLA-A24, A26, or B61 in a positive case, A26, or B61 molecules from 9-10 amino acid of the CMV protein origin was shown, and CD8+CTL reacted to it started growth. In order to help growth of CD8+CTL, IL-2 were added to culture medium. (2) From the specific CD8+CTL stock, the CD8+CTL clone was established at HCMV in which the specific CD8+CTL clone carried out establishment establishment using limiting dilution to HCMV.

[0046] 3. The ERIS pot assay 96 hole MultiScreen-HA plate (Millipore) was coated with 4 degrees C by the anti-IFNgamma monoclonal antibody (R&D Systems, Minneapolis, MN) overnight. After washing each hole by PBS, each cell of T2-A24, and A26 and B61 was wound around each hole. After putting each epitope candidate peptide compounded in the example 1 into each hole and putting at a room temperature for 30 minutes, specific CD8+CTL was added to a suitable number of CMV(s), and it cultivated in the 37-degree C 5% CO2 incubator for 20 hours. CD8+CTL reacted to an epitope candidate peptide secreted IFN to this incubation period. after washing a plate by the 0.05%Tween-20 addition PBS on the next day, it was made to each every 90 react at a room temperature in order of an anti-IFNgamma rabbit blood serum (Genzyme, Cambridge, MA) and a peroxidase label anti-rabbit IgG goat blood serum 0.1M which contain 3-amino-9-ethylcarbasole (Sigma) and 0.015% of H2O2 in a pan Put the sodium acetate buffer (pH 5.0) into each hole, it was made to react at a room temperature for 40 minutes, and the IFNgamma spot was visualized. The spot was counted with the stereoscopic microscope. The actual IFNgamma spot film which used T2-A24 as the antigen presenting cell is shown in drawing 6 A. Moreover, the result of having counted the IFNgamma spot produced in response to the epitope candidate peptides of each under the stereoscopic microscope is shown in drawing 6 B. A bar graph shows the number of spots per 1well. The peptide numbers 60, 81, and 83 correspond to the array numbers 30, 23, and 24 of this invention among drawing, respectively.

[0047] Furthermore, in order to check that the process of the CD8+CTL epitope peptide is carried out to endogenous, the vaccinia virus which discovers pp65 of the vaccinia virus which discovers gB protein of HCMV and HCMV, and HCMV was infected with the skin fibroblast of HLA-A24 positivity, this was made into the antigen presenting cell, and ERIS pot assay made to react to the peptide (pp65 origin of HCMV) of the array number 23 with a specific CD8+CTL clone was performed. The result is shown in drawing 7 . In addition, the result to which the skin fibroblast of HLA-A24 positivity with which the virus is not infected, and a CTL clone were made to react is shown by the inside Mock of drawing. Moreover, the bar graph of each item shows the dilution train of the CD8+CTL clone per 1well, and 200, 100, 50 and 25 from the left. Consequently, the CD8+CTL clone specific to the peptide of the array number 23 reacted only to the HCMV infected cell and pp65 manifestation vaccinia virus infected cell. According to the above methods, the CD8+CTL epitope peptide of the following array numbers 23-32 with which specific CD8+CTL and its CD8+CTL clone reacted to HCMV was obtained.

[0048]

Gln Tyr Asp Pro Val Ala Ala Leu Phe (array number 23)
Gln Tyr Asp Pro Val Ala Ala Leu Phe Phe (array number 24)
Val Glu Leu Arg Gln Tyr Asp Pro Val Ala (array number 25)
Asp Val Pro Ser Gly Lys Leu Phe Met (array number 26)
Asp Val Ala Phe Thr Ser His Glu His Phe (array number 27)
Asp Thr Asp Glu Asp Ser Asp Asn Glu Ile (array number 28)
Asp Leu Leu Leu Gln Arg Gly Pro Gln Tyr (array number 29)
Asn Tyr Leu Asp Leu Ser Ala Leu Leu (array number 30)
Gln Tyr Arg Ile Gln Gly Lys Leu (array number 31)
Gln Tyr Arg Ile Gln Gly Lys Leu Glu Tyr (array number 32)

[0049] It dissolved respectively and filtration sterilization was carried out so that it might become the last concentration of 20mg/ml about the peptide of the array numbers 1-32 at the [example 5] vaccine injections DMSO. 1ml carried out distributive-pouring sealing at a time, and the obtained peptide content solution was made into vaccine injections at the sterilization vial bottle.

[0050] The lymphocyte was separated into HCMV which consists of measuring [example 6] cytokine from the adult peripheral blood of the five specific quantum methods of CD8+CTL, and it stimulated using the peptide of the array number 23 of the HCMV origin, and the HLA-A24 joint peptide of the AIDS virus (HIV) origin as electronegative control. IFN-gamma by which production are recording was carried out was dyed intracellular by the FITC labelled antibody. CD69 which is the activation marker of a T lymphocyte was dyed coincidence by PE labelled antibody. The dyed cell was measured with flow cytometer (BEKUTON-DEIKKINSON, FACScan). The result is shown in drawing 8 . % shown in the upper right of each graph shows the rate of CD8+CTL reacted to a peptide among drawing. It is clear from this result that this method can detect epitope peptide reactivity CD8+CTL specifically.

[0051] The recombination protein manifestation system by the preparation Escherichia coli of the specific CD8+ preparation MHC of the method 1.MHC-tetramer which carries out the quantum of the CTL (1) was used for HCMV in peripheral blood using the MHC-tetramer prepared from the [example 7] CD8+CTL epitope peptide, and HLA A*2402 heavy chain and beta 2-microglobulin were created and refined in large quantities. In addition, the amino acid sequence which a biotin ligase recognizes was beforehand added to the HLA A*2402 heavy-chain C terminal.

Purification HLA A*2402 heavy chain and beta 2-microglobulin were dissolved in 8M urea, respectively. 200 ml refolfing buffer Inside (pH8.0; 100 mM Tris-HCl, 400 mM L-arginine-HCl, 2 mM EDTA, 0.5 mM oxidative glutathione, 5 mM reduced glutathione), it injected with each using the syringe to which 27 gage needles were attached peptide 12mg of the array number 23, 18.6mg of A*HLA 2402 heavy chains, and 13.2mg of beta 2-microglobulin. After agitating in 10-degree-C thermostat for 48 to 72 hours and urging formation of MHC, refolfing buffer containing MHC was dialyzed in 4-degree-C thermostat for 24 hours to distilled water of 1.8L, and refolfing buffer after dialysis was condensed to 2ml using cent RIPUREPPU 10 (MILLIPORE, Bedford, MA). Superdex 200 HR MHC which flows into per molecular weight 45KD with the gel filtration chromatography using a column (Amersham Pharmacia Biotech AB, Uppsala, Sweden) was isolated.

[0052] (2) The biotin was combined with the specific part of a HLA A*2402 heavy-chain C terminal using the preparation biotin ligase (AVIDITY, Denver, CO) of biotin-ized MHC. Superdex 200 HR MHC which added the biotin with the gel filtration chromatography using a column was refined.

[0053] (3) Preparation PE indicator streptoavidin of an MHC-tetramer (Molecular Probe, Eugene, OR) Purification biotin-ized MHC was mixed by the mole ratio 1:4. Superdex 200 HR The MHC-tetramer containing the array number 23 which flows into per molecular weight 480KD with the gel filtration chromatography using a column was isolated. It condensed [ml] in about 3mg /using Centricon 10 (MILLIPORE), and saved at 4 degrees C. NaAzide, EDTA, Leupeptin, and Pepstatin were added as a preservative.

[0054] 2. The peripheral blood lymphocyte (2x106) separated into HCMV using an MHC-tetramer from the adult infected with the specific quantum HCMV epitope peptide specific CD8+ T cell clone (2x105) or specific HCMV of CD8+CTL was suspended in the 2%FCS content PBS of 50microL within 1.5ml Eppendorf tube. It adds each an MHC-tetramer and FITC indicator anti-CD8 antibody (CALTAG, burlingame, CA) every [1micro / L], and it put on this gently for 15 minutes, and it was made to react to 37-degree-C thermostat. In 2% FCS content PBS 1ml, the cell after a reaction was washed 3 times and, subsequently to the paraformaldehyde content PBS (1ml), was suspended 0.5%. CD8+CTL combined with the MHC-tetramer containing the array number 23 was dyed by indicator coloring matter, counted the tetramer positivity cell in a FITC-CD8+ T cell using FACS Calibur (BEKUTON DEIKKINSON), and calculated %.

[0055] The result of analysis is shown in drawing 9 . Since CD8+CTL specific to HCMV combined with the MHC-tetramer is dyed, it moves to the right. A is an EBV specific CD8+CTL clone (electronegative control) among drawing, and B is a CD8+CTL clone (positive control) specific to the peptide of the array number 23. C is HCMV antibody negative adult peripheral blood, and D is HCMV antibody-positive adult peripheral blood (the ensemble of the point at the upper right of drawing D is a tetramer positivity cell). It is clear from this result that the MHC-tetramer containing the array number 23 is dyeing HCMV reactivity CD8+CTL specifically.

[0056]

[Effect of the Invention] EBV or HCMV can be provided with a specific CD8+CTL epitope peptide by this invention. Moreover, this epitope peptide can be used, and infection of EBV or HCMV can be managed, treated or prevented. Furthermore, it is possible to carry out the quantum of the specific CD8+CTL to EBV or HCMV.

[0057]

[Layout Table]

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[Translation done.]

胞中のテトラマー陽性細胞をカウントし、%を計算した。

【００５５】解析の結果を図９に示す。MHC-テトラマーに結合したHCMVに特異的なCD8⁺CTLは染色されるので右に移動する。図中、AはEBV特異的CD8⁺CTLクローン（陰性コントロール）であり、Bは配列番号23のペプチドに特異的なCD8⁺CTLクローン（陽性コントロール）である。CはHCMV抗体陰性成人末梢血であり、DはHCMV抗体陽性成人末梢血（図Dの右上の点の集団がテトラマー陽性細胞）である。この結果から、配列番号23を含むMHC-テトラマーがHCMV反応性CD8⁺CTLを特異的に染色していることが明らかである。

【００５６】

【発明の効果】本発明により、EBV又はHCMVに特異的なCD8⁺CTLエピトープペプチドを提供することができる。また、該エピトープペプチドを用いてEBV又はHCMVの感染を管理、治療又は予防することができる。さらに、EBV又はHCMVに特異的なCD8⁺CTLを定量することが可能である。

【００５７】

【配列表】

SEQUENCE LISTING

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【図面の簡単な説明】

【図1】CD8⁺CTLによるウイルス感染細胞の認識機構を示す図である。図中、CD8⁺CTLエピトープペプチドは本発明のペプチドである。

【図2】MHC-テトラマーの調製方法を示す図である。

【図3】MHC-テトラマーとEBV又はHCMV特異的CTLの結合

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

[Drawing 1] It is drawing showing the recognition device of the virus infection cell by CD8+CTL. A CD8+CTL epitope peptide is a peptide of this invention among drawing.

[Drawing 2] It is drawing showing the preparation method of an MHC-tetramer.

[Drawing 3] an MHC-tetramer, EBV, or HCMV -- specific -- it is drawing showing association of CTL.

[Drawing 4] It is drawing showing the preparation method of an MHC-MAG bead.

[Drawing 5] EBV by the MHC-MAG bead, or HCMV -- specific -- it is drawing showing the isolation of CTL.

[Drawing 6 A] It is drawing showing the photograph of the IFNgamma spot in the example of identification of a CD8+CTL epitope peptide specific to HCMV by ERIS pot assay.

[Drawing 6 B] It is drawing showing the spot count result in the example of identification of a CD8+CTL epitope peptide specific to HCMV by ERIS pot assay.

[Drawing 7] It is drawing showing that the process of the identified epitope peptide is carried out to endogenous, and infection cell surface is shown it.

[Drawing 8] It is drawing showing the example of a quantum of the CD8+ T lymphocyte reacted to the peptide which made IFN-gamma the index.

[Drawing 9] It is drawing showing the example of identification of a specific CD8+ T lymphocyte in HCMV by the tetramer prepared with the epitope peptide of this invention.

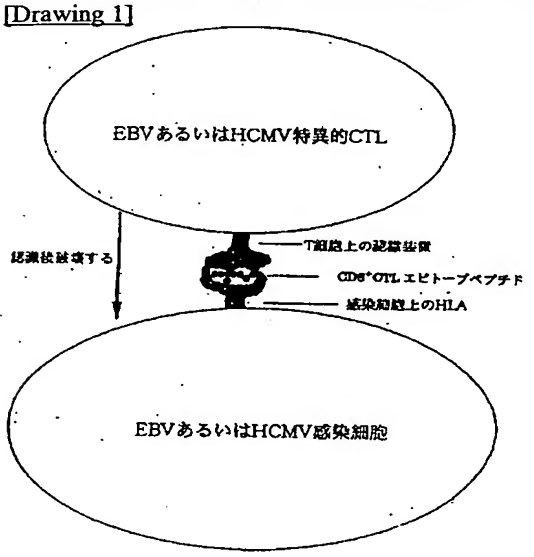
[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DRAWINGS

[Drawing 1]



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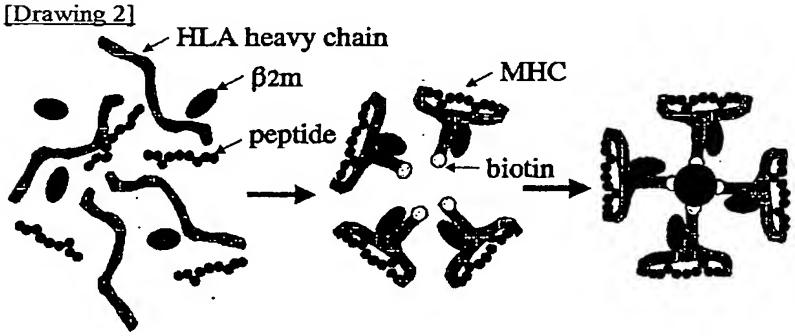
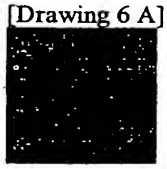


大腸菌による組換え蛋白産生と *in vitro* refolding

MHCのビオチン化

蛍光標識アビジンによる4量体化

[Drawing 6 A]



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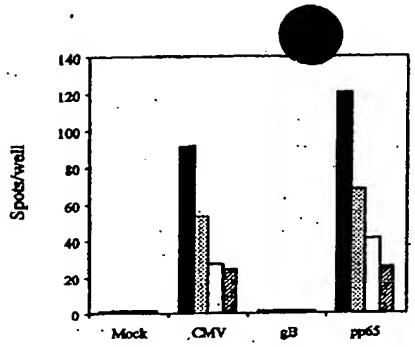

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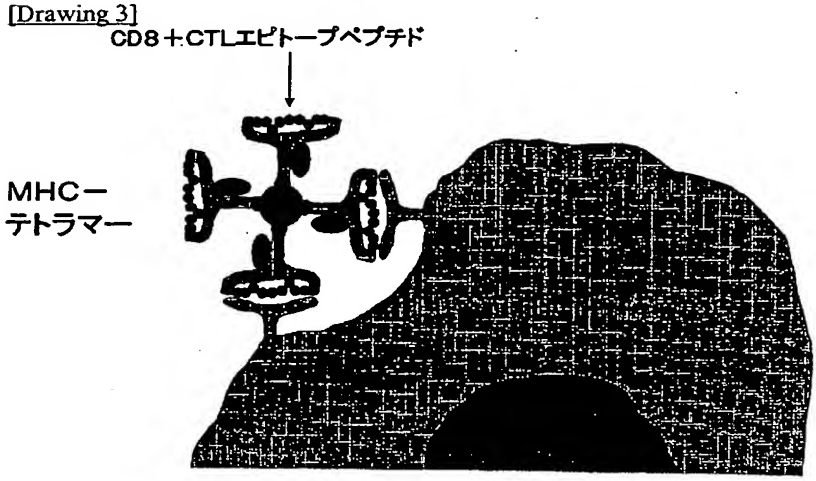

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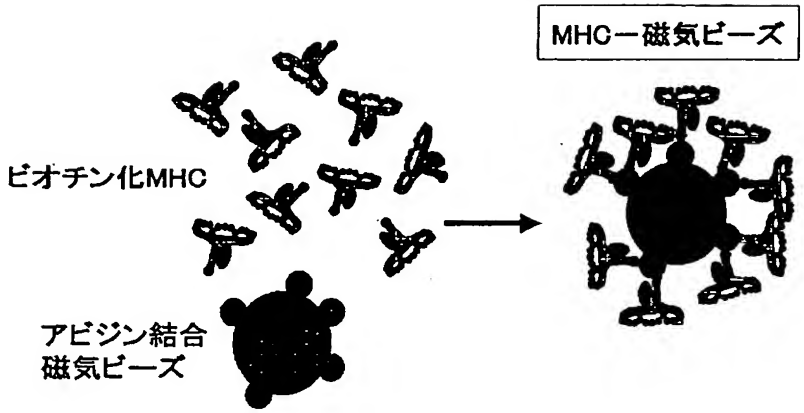

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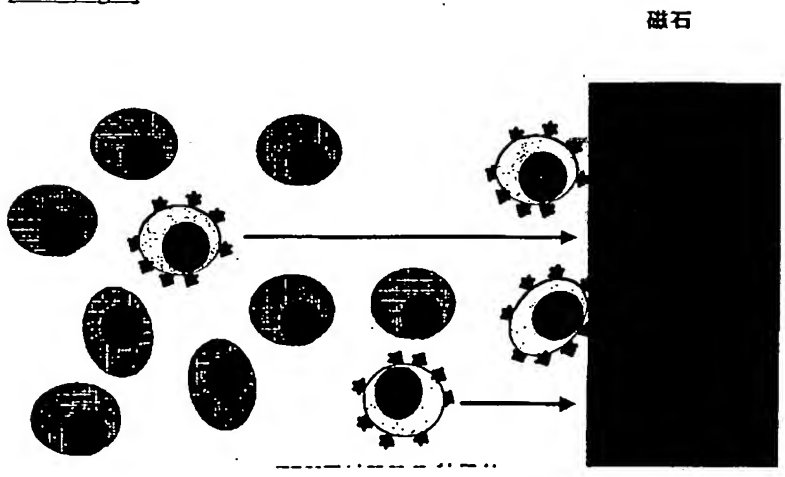
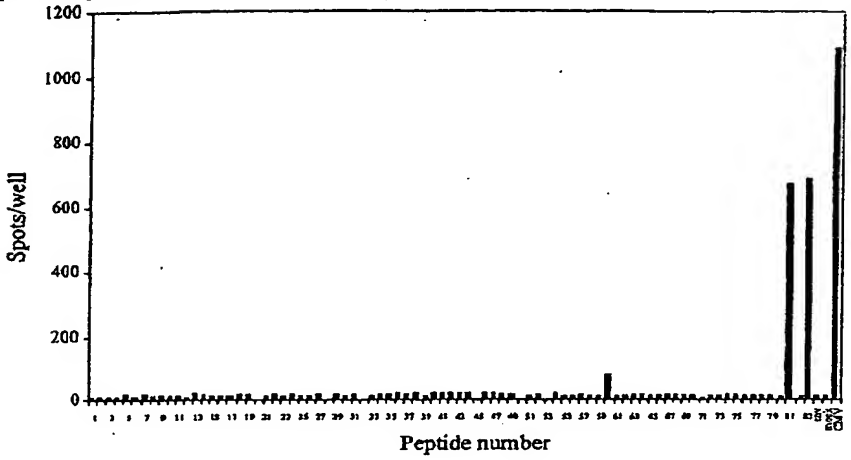


EBV又はHCMV特異的CTL [redacted]

[Drawing 6 B]



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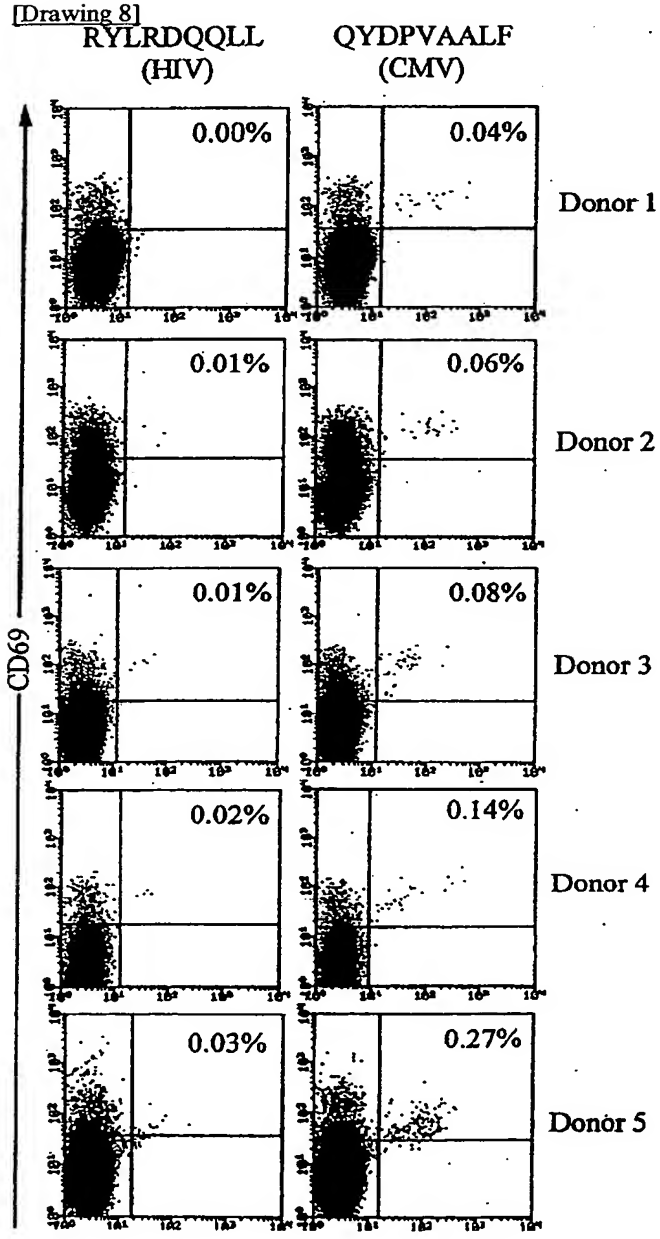
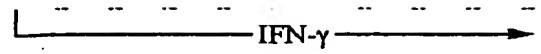
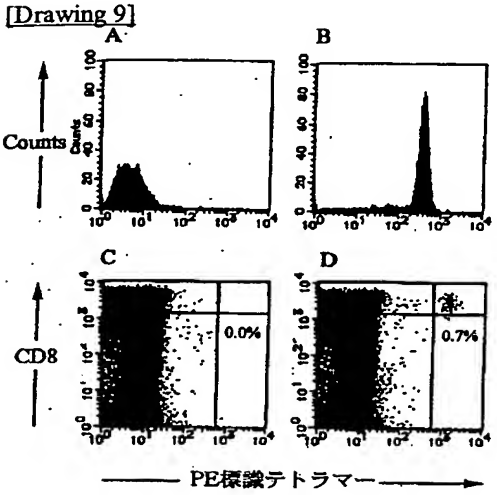




[Drawing 9]



[Translation done.]